SIZE AND MORPHOGENESIS IN THE BUD OF BOTRYLLUS

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The bud of *Botryllus* first appears as a disc arising in the anterior wall of each of the atrial chambers. The disc grows a little and then transforms into a sphere. The size to which the disc grows before it starts to transform varies among different bud generations in a *Botryllus* colony, increasing slightly with each successive generation of buds. The present account is primarily a comparison of the development of the buds arising from the smaller discs of early generations with the large discs of late generations, within the colony formed from a single fertilized egg. In addition to differences in size of bud primordia from early and late generations in the colony, there are usually size differences between the bud primordia of the right and left sides, that of the right side being the larger.

The development of a bud has as its basis a continuous material expansion from the small group of cells constituting the primary disc to a functional bud of several thousand times its volume and cell number. The significance of this expansion is paramount. As the disc expands in area it becomes curved into a hollow sphere. As the sphere expands, its surface folds inwards to divide it into three chambers, the major territories of the body. With continued expansion, further surface folding occurs to divide off smaller territories such as neural mass, heart, and intestine. It can be said that for each successive size the material (mass, area, cell number, etc., however it may be expressed) present at that moment expresses virtually every character of the final organization that is not inhibited by the limitation of size itself.

Each bud disc arises from the atrial epithelium as a group of cells that gradually acquire a columnar form. The epidermis forms an equivalent overlying component of the disc, but plays a relatively minor part in the subsequent development. Since every disc of atrial cells has to develop from the general atrial epithelium, there is almost certainly no real minimum size that can be compared in different generations. On the other hand, the disc in every case grows to a certain extent before changes in form begin, and the size or cell number of the disc at its maximum size, which is a precise stage, is a value readily compared.

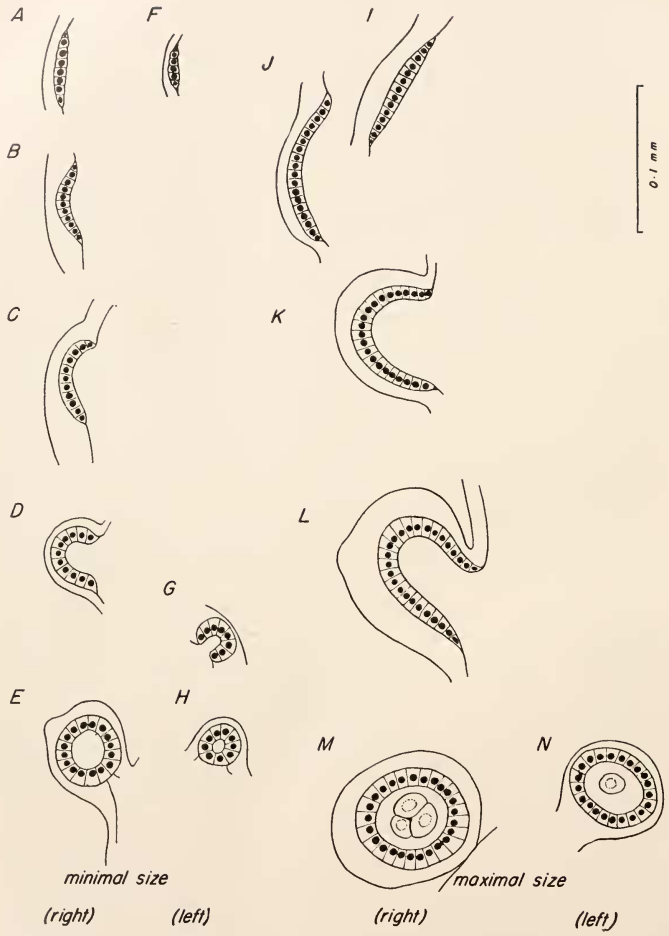

Fig. 1. Formation of bud vesicles, all in optical section. *A*, *F*, and *I* are three maximal bud discs. *A–E* represents vesicle formation from small right maximal disc from zoöid of young colony. *F–H*, the smaller left maximal disc of same series. *I–M*, vesicle formation from large right maximal disc from zoöid of a mature colony. *M* and *N*, right and left vesicles from same individual and forming three and one mature ova respectively.

The smallest maximal disc commonly seen consists of about six cells in optical section, the largest of about fourteen cells, or a difference of about eight times in volume of tissue or number of cells present at this stage. Figures 1, 2, and 3 represent a comparison of the development of two sizes of maximal right bud discs, from early and late generations respectively. In Fig. 1, *G, H,* and *N,* the smaller left buds are also shown. In this figure several features of comparative interest are clear. The relative difference in size of three maximal disc stages is maintained in the subsequent stages of hemisphere and sphere. In optical section these maximal discs have 5, 8, and 14 cells respectively (Fig. 1, *F, A* and *I*), representing totals of about 21, 48, and 150 cells (ratios 1 : 2·1 : 7·1). In the corresponding closed sphere or vesicle stages, optical sections show 9, 15, and 25 cells respectively (Fig. 1, *H, E* and *M*), representing cell totals of about 33, 75, and 210 (ratios 1 : 2·2 : 6·3). From these values two facts emerge. The ratio of cell numbers representing the smallest and largest maximal disc illustrated is about 1 : 7. The same ratio holds for the closed vesicle stage, and it is evident that whatever the size of the maximal discs, the transformation is correlated with an increase in cell number of about one and one-half times that of the disc.

Morphogenesis is thus independent of absolute cell number, but closely dependent on relative cell number.

In Fig. 1 two other features are evident. The relation of morphogenesis to cell number is the same in the epidermis as in the atrial tissue. The epidermis conforms in size and shape to the inner tissue, and as may be seen in Fig. 1, *E* and *K,* the protrusion foreshadowing the ventral ampullary vessel appears in both small and large vesicle stages, and in spite of the very early stage in development as a whole.

It is of greater interest that gonad tissue is segregated from the right and left lateral wall of the vesicle stage in the large forms but not in the small. These two correlated variations in the vesicle stage, namely degree of segregation of gonad tissue and the absolute size, produce an increasingly marked effect on later stages of development. Figure 2 shows immediately succeeding stages drawn to the same scale as those in Fig. 1. Figure 3 shows still later stages at necessarily reduced scales. In each case equivalent stages are shown for the development of both large and small primordia.

The two primary differences, in size and gonad development, are maintained in an increasingly obvious form. Thus in Fig. 2, the three stages *A, B,* and *C* are morphologically equivalent to the stages *D, E,* and *F.* In *A* and *D* the folding of the vesicle wall to delimit the primary divisions of the body are just beginning. In *B* and *E* they are com-

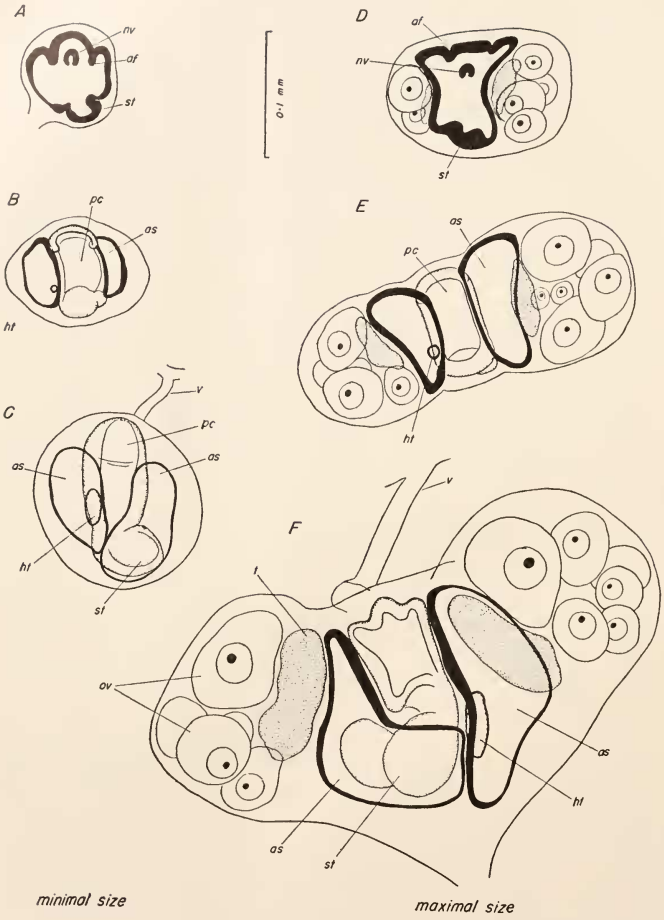

FIG. 2. Development, at same magnification, of series *A–E* and *I–M* of Fig. 1. *A–C*, development of small vesicle, *D–F* of large vesicle, showing differences in size of equivalent stages and in presence and absence of gonads.

af, atrial folds; *as*, atrial sac; *ht*, heart; *nv*, neural vesicle; *ov*, ova; *pc*, pharyngeal cavity; *st*, stomach; *t*, testis; *v*, ventral ampullary vessel.

pleted, and atrial chambers, pharynx, intestine, heart, and neural mass already exist as unit regions. The difference in diameter of the closed vesicle stage shown in Fig. 1, *E* and *M*, is fully maintained. In addition there is the striking difference in gonad development. In the series of stages associated with the small primordium none appear, in the larger they continue to develop and become massive organs on each side between the lateral wall of the atrial epithelium and the epidermis. These differences become more and more pronounced, as may be seen in Fig. 3.

Size and General Organization

While the structural consequences of the primary difference in size become progressively more obvious, one feature needs to be emphasized strongly. The great difference in cell number constituting maximal disc stages is maintained at least in the later closed vesicle stage, the increase being about one and one-half times. The difference in cell number is expressed less markedly in disc and vesicle diameters. In the two main series illustrated in Fig. 1, the diameter of the larger series is about one and two-thirds that of equivalent stages of the smaller series. Excluding gonads for the time being, this difference in linear dimension of the two series is maintained closely in the later stages shown in Figs. 2 and 3 up to and including the active functional stage. Not only is the linear size difference maintained throughout development, but it is equally expressed in the number of such multiple structures as stigmata. In the stages in which stigmata are just becoming perforate and in which they are active organs, the number of rows of stigmata is six in the smaller buds, ten or eleven in the larger, while the number of stigmata per row in the smaller is 12 and in the larger 22. The number of stigmata does not change during development. Thus in the two series the number of stigmata formed is proportionate to size, since both linear dimension of the whole, and the number of rows of stigmata and number of stigmata per row, vary as one to about one and two-thirds.

The difference in whole size of equivalent stages, which is expressed numerically in multiple organs such as stigmata, applies equally to organ size. This is the case for the heart, for in the three stages—primary heart vesicle, initial beating, and final—the same relative size differences are maintained.

Since the relative difference in size between the two series is maintained virtually at a constant level for all stages, it follows that each stage represents a certain degree of expansion in terms of a preceding stage, whatever may be the absolute size. A specific degree of expression of the complete organization is correlated with a certain size or

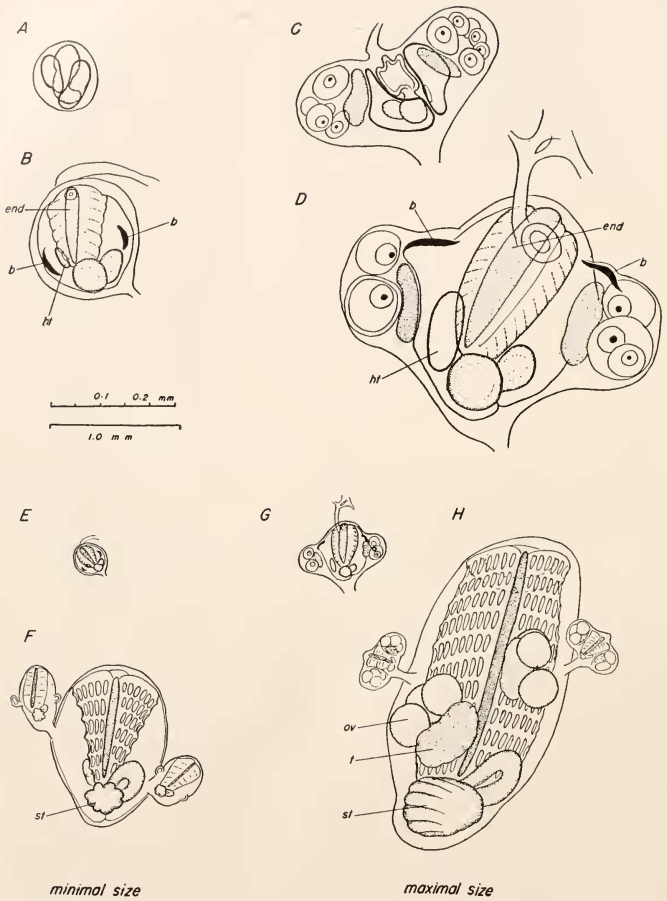

Fig. 3. Continuation of same two developmental series, at two smaller magnifications. *A–B, E–F* continued development of smaller bud disc and vesicle shown in Figs. 1 and 2. *C–D,* and *G–H,* continued development of larger vesicle.

For purposes of comparison, *A* and *C* represent at the lower magnification the stages shown in Fig. 2, *C* and *G;* in the same way *E* and *H* are reduced from *B* and *D*. *B* and *D* are equivalent stages and have formed maximal bud discs. *E* and *H* are also equivalent and have become active zoöids. The difference with regard to size, presence of gonads, and length and number of rows of gill slits is obvious.

b, bud disc; *end,* endostyle; *ht,* heart; *ov,* ova; *st,* stomach; *t,* testis.

material quantity. This size is not absolute and is not expressable in actual measurement or cell number, but must be expressed in terms of reference to the absolute size of the maximal disc stage. This is highly significant and will be referred to again.

Development of Gonads and Initial Size

Confining the present account to the two extremes already illustrated, there is a spectacular difference in the condition of the gonads in the two series, one producing mature gonads and the other none. This difference goes back to the first stages of development. In the larger series, the gonads are separated or extruded from the lateral walls of the primary vesicle even before closure is complete. Once separated, the gonads develop apparently as independent unit regions. The separation phase is comparatively brief, and there is no tendency to form gonads except during this precise phase of the whole development. The ova destined to become mature are the first tissue to be separated, testis and prospective immature ova separating a little later.

In the smaller series, no separation of gonads occurs at all during the equivalent phase, and no gonads appear at any later time. Consequently the massive lateral bulges representing the growing gonads in the developing buds of mature colonies are absent altogether in those of very young colonies.

The correlation of presence or absence of gonad separation (and therefore of subsequent development) with the size of the transforming disc stages suggests at once that size itself may be the determiner.

Gonad tissue is separated during a very definite and specific period of bud development, namely, during a period starting before closure of the primary vesicle and lasting until the vesicle is more or less subdivided into its three primary regions. At no other moment in development, either earlier or later, is there any indication of gonad formation. There is no absolute proof that at no other time under any conditions can gonad tissue be separated, but it is reasonably certain that the capacity to produce gonad tissue is definitely limited to the period or phase in which production always occurs. In other words, the gonad primordium is determined and formed at as precise a period in the whole development as is the case for other organs, such as the heart.

Accordingly, if this assumption is made, it is easy to account for the suppression of gonad formation in the development of small buds. The situation is clearest if one compares the largest and smallest of the four vesicle stages shown in Fig. 1. In the largest, three prospective mature ova have separated from the vesicle wall. The extrusion of these par-

ticular cells commences immediately after the attainment of the open hemisphere stage. The gonad material thus separated must represent a certain minimum proportion of the lateral wall from which it arises, of the order of one-quarter to one-half. It is separated when the vesicle cells total about 160 and when there are about 22 to 26 cells in optical section (Fig. 1, *M*). At this stage, in other words, the material from which ova are separated is in the form of a sufficient number of cells for individual cells representing individual ova to be pushed out.

In the equivalent stage of the smallest series, only 7 or 8 cells constitute the optical section, and the region from which gonads and atrial wall should be differentiated consists, on each side, of only 2 cells in optical section. Accordingly the region constituting the prospective ova is at this stage and size inadequately cellular and the separation of ova at this moment becomes mechanically impossible.

Gonad formation, however, is not momentary but occupies a period of time. In the smallest series even the late closed vesicle stage consists of so few cells (Fig. 1, *E*) that at no time during the proper period can gonad tissue be separated. In the largest series the prospective mature ova are extruded before closure, male tissue and prospective immature ova during and shortly after closure of the vesicle, and some additional male cells after completion of the extrusion of female cells.

In series of developing buds of intermediate size all conceivable types of immature gonads should be found. This is the case. In a series slightly larger than the smallest, a sufficient size or cell number is attained before the gonadial phase is completely passed, and some male tissue is separated at the end of that phase. In a somewhat larger series again, and a little sooner, more gonad tissue is separated consisting of prospective immature ova and male tissue sufficient to develop into a lobular testis of submaximal size. Similarly, in a larger but not maximal series, there may be a separation at or before closure of one or two or three prospective mature ova. In other words, a complete grading from none to mature 4-ova gonads exists, correlated both with size of series and with time of separation.

Conclusions and Summary

In successive bud generations within a colony there is a progressive increase in the size of the bud rudiments and zoöids subsequently developing from them. Dealing with material derived from a single fertilized egg, it is possible to determine the relationship or importance of rudiment size to morphogenesis. Development in every case is simple and direct. It consists of the growth of the rudiment to a maximal

disc stage, the conversion of the disc into a sphere, the subdivision of the sphere or vesicle into unit regions, the whole process being accompanied and conditioned by expansion or growth of tissue.

The maximal disc is a precise relative stage. The larger discs, typical of late and mature generations, may be seven times as large in area as those of early generations.

Whatever the size of the maximal disc, succeeding stages bear to it definite growth ratios. A certain percentage expansion or growth of the disc tissue is correlated with a specific developmental stage, whatever the absolute sizes may be. Absolute size must be determined during the initial phase of development before the disc exhibits any tendency to transform into a vesicle.

Ultimate size being thus initially determined, there is variability in the following expressions of size. All organs and regions vary in absolute size, maintaining their proportionate dimensions relative to the whole. Multiple structures, such as stigmata, vary little in absolute size for a given stage but vary in number in proportion to tissue area. The relatively massive gonads, fully formed only in the largest series, are partially or completely inhibited in the smaller.

Gonad formation is essentially a serial separation of the various components during a short phase of development, lasting from the open hemisphere stage to the expanded closed vesicle stage. If the size of the whole permits separation of each component as discrete cells at the proper time for separation, maximal mature gonads will be formed and develop. If size is so reduced that the various components cannot be materially separated as cells, separation is inhibited and no gonads will develop at the normal or any other time. With successive increases in size from this last condition an adequate cellular state is reached, at first including the later phases of the gonadial period and progressively including the earlier, so that a series of immature gonads appear in the inverse order of normal maximal development. Prospective mature ova do not appear at a time normal for the appearance of prospective immature ova or for male cells. Gonad components that do not separate at their normal time do not appear at all.